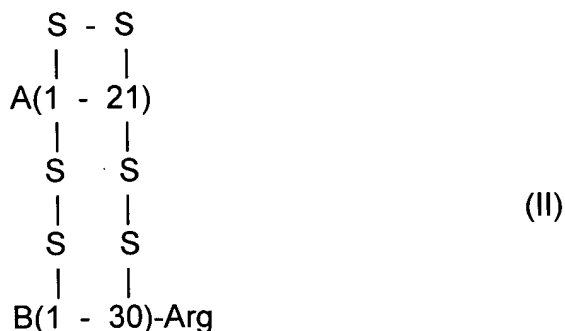


Petition for a One-Month Extension of Time and the required fee. Kindly enter the following amendment:

IN THE CLAIMS:

Please amend claims 21-22, 25-26, and 31 as follows:

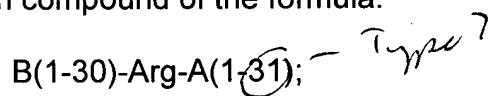
21. (Twice Amended) A method for the preparation of a mono-Arg-insulin compound of formula II



in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, which comprises:

- (a) expressing in a bacterium a DNA molecule encoding a fusion protein

which comprises a mini-proinsulin compound of the formula:



- (b) liberating said mini-proinsulin compound from said fusion protein;
(c) folding and forming disulfide bridges in said mini-proinsulin compound;
(d) incubating said mini-proinsulin compound with trypsin [at a pH of about

6.8 to produce mono-Arg-insulin, under conditions where no crystals are formed;
followed by]; and

- (e) precipitating the mono-Arg-insulin.

22. (Twice Amended) A method for the preparation of insulin which comprises:

(a) expressing in a bacterium a DNA molecule encoding a fusion protein

which comprises a mini-proinsulin compound of the formula:



in which B(1-30) and A(1-21) denote the B and A chains of insulin;

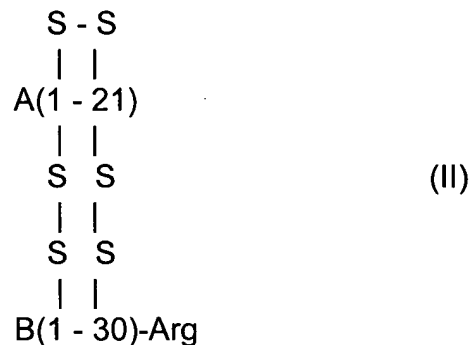
(b) liberating said mini-proinsulin compound from said fusion protein;

(c) folding and forming disulfide bridges in said mini-proinsulin compound;

(d) simultaneously incubating said mini-proinsulin compound with trypsin and carboxypeptidase B [at a pH of about 6.8 to produce insulin, under conditions where no crystals are formed; followed by]; and

(e) precipitating the insulin.

25. (Amended) A method for the preparation of a mono-Arg-insulin compound of formula II



in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, which comprises:

(a) expressing in a bacterium a DNA molecule encoding a fusion protein

which comprises

B(1-30)-Arg-A(1-21)

bonded via a bridging member,

- Met - Ile - Glu - Gly -Arg - ,

to a peptide which stabilizes the fusion protein;

(b) liberating a mini-proinsulin compound from said fusion protein by cleaving the expressed fusion protein resulting from step (a) with cyanogen bromide;

(c) folding and forming disulfide bridges in said mini-proinsulin compound;

(d) incubating said mini-proinsulin compound with trypsin [at a pH of about 6.8 to produce mono-Arg-insulin, under conditions where no crystals are formed; followed by] ; and

(e) precipitating the mono-Arg-insulin.

26. (Amended) A method for the preparation of insulin which comprises:

(a) expressing in a bacterium a DNA molecule encoding a fusion protein which comprises

B(1-30)-Arg-A(1-21)

bonded via a bridging member,

- Met - Ile - Glu - Gly -Arg - ,

to a peptide which stabilizes the fusion protein;

(b) liberating a mini-proinsulin compound from said fusion protein by cleaving the expressed fusion protein resulting from step (a) with cyanogen bromide;

LAW OFFICES

FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000

02
Cont

(c) folding and forming disulfide bridges in said mini-proinsulin compound;

(d) simultaneously incubating said mini-proinsulin compound with trypsin and carboxypeptidase B [at a pH of about 6.8 to produce insulin, under conditions where no crystals are formed; followed by] ; and

(e) precipitating the insulin.

03

31. (Amended) A method for the preparation of insulin, without formation of substantial amounts of insulin Des-B30, comprising:

(a) expressing in a bacterium a DNA molecule encoding a fusion protein which comprises

B(1-30) - Arg - A(1-21)

bonded via a bridging member,

-Met-Ile-Glu-Gly-Arg-,

to a peptide which stabilizes the fusion protein;

(b) liberating a mini-proinsulin compound from said fusion protein by cleaving the expressed fusion resulting from step (a) with cyanogen bromide to produce mini-proinsulin;

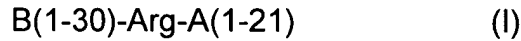
(c) incubating the product formed in step (b) with sodium tetrathionate to form hexa-5-sulfonate;

(d) simultaneously incubating the S-sulfonate mini-proinsulin formed in step (c) with trypsin and carboxypeptidase [at a pH of about 6.8 under conditions where no crystals are formed]; and

(e) precipitating the insulin.

Please add the following new claims:

--33. A compound of the formula I



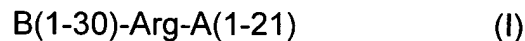
wherein A(1-21) and B(1-30) denote the A and B chains of human insulin.

34. A nucleic acid sequence encoding the compound of formula I as claimed in claim 33.

35. A vector comprising the nucleic acid sequence of claim 34.

36. A host cell containing the nucleic acid sequence of claim 34.

37. A fusion protein comprising a compound of the formula I



wherein A(1-21) and B(1-30) denote the A and B chains of human insulin, and wherein the compound is bonded via a bridging member



to a peptide which stabilizes the fusion protein.

38. A process for the preparation a compound as claimed in claim 33, which comprises:

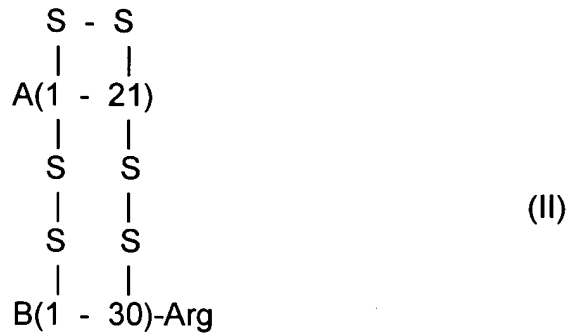
a) expressing a DNA sequence encoding the compound of the formula I in a bacterium; and

b) when the DNA sequence encodes a fusion protein, liberating the compound of formula I from the fusion protein.

39. A method for the preparation of a compound of the formula II

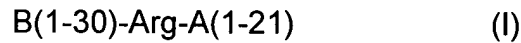
LAW OFFICES

FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-406-4000



wherein A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, comprising:

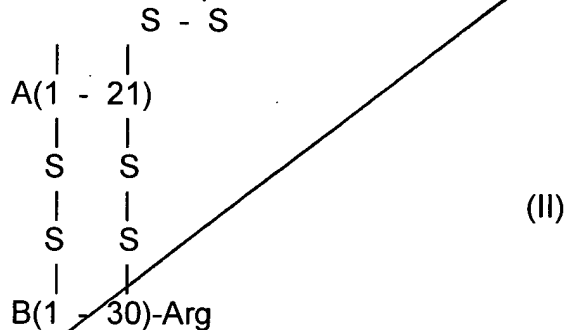
- a) expressing a DNA sequence encoding the compound of formula I



in a bacterium; and

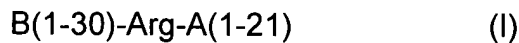
- b) cleaving the expressed compound of step (a) with trypsin.

40. A method for the preparation of a compound of the formula II



wherein A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, comprising:

- a) expressing a DNA sequence encoding the compound of formula I



in a bacterium;

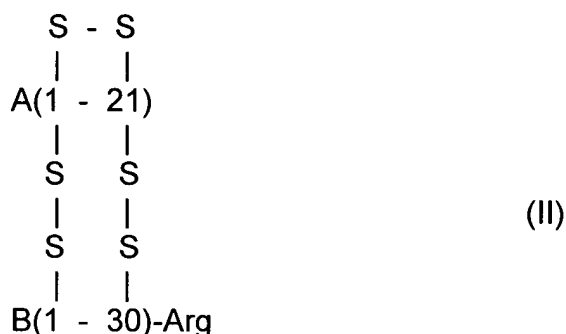
OH
cont
Sub p1

Sub p1
b) cleaving the expressed compound of step (a) with trypsin resulting in the compound of the formula II; and

~~(c) cleaving the resulting compound of step (b) with carboxypeptidase B.~~

41. The method of claim 40, wherein steps (b) and (c) are carried out in one vessel without having to isolate the intermediate compound of the formula II.

Sub p1
42. A method for the preparation of a mono-Arg-insulin compound of the formula II



in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, which comprises:

(a) expressing a DNA sequence encoding a mini-proinsulin compound of the formula:



in a yeast; and

(b) cleaving said mini-proinsulin compound with trypsin.--

REMARKS

Claims 21-23, 25-27, 31, and 33-42 are currently pending in this application.

Support for claims 33-44 can be found in the specification as a whole and pages 2-5